

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization International Bureau



(43) International Publication Date
10 March 2005 (10.03.2005)

PCT

(10) International Publication Number
WO 2005/021021 A1

(51) International Patent Classification⁷:

A61K 35/78

(74) Agent: MAC PATENT & LAW FIRM; 5th Floor, Hwaseung Bldg., #15-3, Yangjae-Dong, Seocho-Ku, Seoul 137-130 (KR).

(21) International Application Number:

PCT/KR2003/001743

(22) International Filing Date: 28 August 2003 (28.08.2003)

(25) Filing Language:

English

(26) Publication Language:

English

(71) Applicant (for all designated States except US):
PURIMED INC. [KR/KR]; 4F Woosung B/D, 678-5, Yeoksam-dong, Kangnam-gu, Seoul 135-915 (KR).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **SHIN, Min-kyu** [KR/KR]; 312-99, Hwicheong-dong, Dongdaemoon-gu, Seoul 130-090 (KR). **HONG, Moo-chang** [KR/KR]; 22-1601 Miseong A.P.T., 408, Apgujeong-dong, Gangnam-gu, Seoul 135-110 (KR). **BAE, Hyeon-su** [KR/KR]; 503-202 Mido2cha A.P.T., Banpo-dong, Seocho-gu, Seoul 137-040 (KR). **YOON, Jeong-hwan** [KR/KR]; 5-1006 Samho A.P.T., Indeokwon 442-2, Poil-dong, Uiwang-si, Gyeonggi-do 437-830 (KR). **KANG, Moon-kyu** [KR/KR]; 301-2202 Buyeong A.P.T., 2-1, Donong-Dong, Namnyangju-si, Gyeonggi-do 472-100 (KR). **OH, Jung-wan** [KR/KR]; 108-1202 Sambu A.P.T., Sujin-dong, Sujeong-gu, Seongnam-si, Gyeonggi-do 461-180 (KR).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

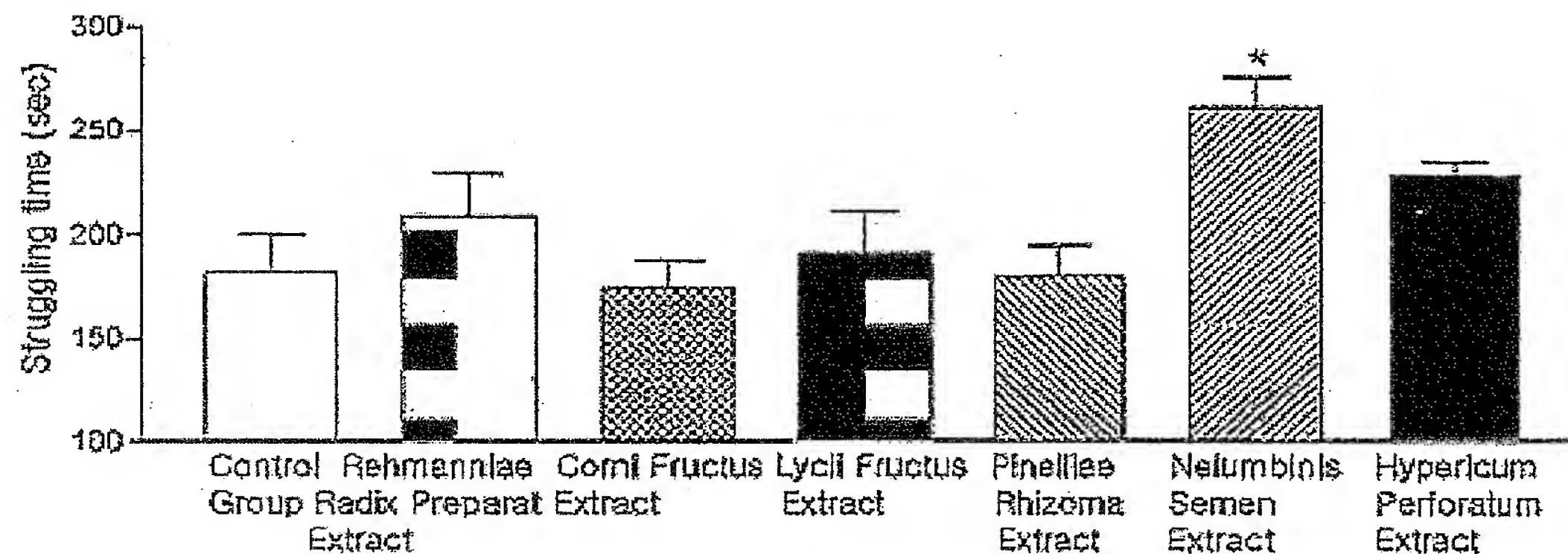
(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- with amended claims and statement

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: EXTRACT OF NELUMBINIS SEMEN FOR THE TREATMENT OF DEPRESSION



(57) Abstract: Disclosed are a Nelumbinis Semen extract for treating depression, a method of preparing the extract, and a pharmaceutical composition and a health food, comprising the extract. The Nelumbinis Semen extract is prepared by extracting Nelumbinis Semen with an alcohol or an alcohol solution. The Nelumbinis Semen extract has been demonstrated using animal behavioral, biochemical and molecular biological methods to have strong antidepressive activity, as well as inhibiting immune suppression caused by depression, leading to normal immune responses, and reducing side effects of conventional antidepressants, thereby ensuring safety. Thus, The Nelumbinis Semen extract is useful for preparing a composition or health food for treating depression.

WO 2005/021021 A1

EXTRACT OF NELUMBINIS SEMEN FOR THE TREATMENT OF
DEPRESSION

[Technical Field]

The present invention relates, in general, to an
5 extract of Nelumbinis Semen (*Nelumbo nucifera*) having a
therapeutic effect on depression, a method of preparing the
extract, and a pharmaceutical composition and a health food
comprising the extract. More particularly, the present
invention relates to a Nelumbinis Semen extract obtained by
10 extracting Nelumbinis Semen with an alcohol or an alcohol
solution and concentrating and drying the resulting extract,
a method of preparing the Nelumbinis Semen extract, and a
pharmaceutical composition and a health food which comprise
the Nelumbinis Semen extract as an effective component.

15 **[Background Art]**

Mental damage occurring in the complicated modern
society is, contrary to in the past, mostly caused by weak
but prolonged and repeated stress from usual activities
rather than large psychological impact or stimuli. Such
20 stress is difficult to be recognized by patients and easily
overlooked during hospital visits by patients, and thus
accumulates, causing individuals to suffer from depression.

Depression is an emotional pathological phenomenon
occurring regardless of objective situations. Emotional

symptoms of depression include depressed behavior during all activities, anhedonia (loss of interest or pleasure), diminished mental capacity, pessimism, poor self-esteem, and suicidal thoughts that occasionally lead to suicide 5 attempts. Physical symptoms of depression include decreased appetite, insomnia, constipation, diminished sexual desire, reduced immune functions, and patients' susceptibility to diseases due to the reduced immune function.

There has been so far no theory that perfectly 10 explains the mechanism causing depression and the action mechanism of antidepressants for treating depression. However, for many years, the prevailing hypothesis is that depression is caused by an absolute or relative deficiency 15 of monoamine neurotransmitters in synapses of the central nervous system, such as serotonin, norepinephrin and dopamine. In this regard, all antidepressants have pharmaceutical action to increase concentrations of neurotransmitters in central serotonin or noradrenaline synapses.

20 Antidepressants are divided into three major types according to the mechanism involving increasing the neurotransmitter levels: tricyclic antidepressants (TCA); monoamine oxidase inhibitors (MAOI); and selective serotonin reuptake inhibitors (SSRI).

25 Monoamine oxidase inhibitors, such as phenelzine developed a relatively long time ago, have a severe adverse

effect of inducing heart diseases, and thus, have not been widely used recently. Tricyclic antidepressants such as imipramine also have anticholinergic, sedative, and other side effects related to the cardiovascular system. Thus, 5 recent research focuses on the development of therapeutic agents against depression using selective serotonin (5-HT) reuptake inhibitors (hereinafter, referred to simply as "SSRI") as antidepressants with fewer side effects. Representative examples include fluoxetine (brand name: 10 Prozac), paroxetine (brand name: Seroxate), and sertraline (brand name: Zoloft), which are widely approved due to their clinical efficacy. However, the aforementioned drugs also have side effects such as whole-body fatigue, sexual dysfunction and insomnia. Administration of antidepressants 15 was reported to typically activate a serotonin receptor by increasing serotonin levels, leading to an activation of PKA that is downstream of the serotonin receptor and eventually increases in protein levels of CREB, brain-derived neurotrophic factor (BDNF) and its receptor, trkB. These increased protein levels are considered to indicate effective 20 actions of antidepressants in molecular levels (*J. of Psychosomatic Research* 53, 687-697 (2002)). In addition, the administration of antidepressants restores to normal levels decreased concentrations of cortisol and IL-2 and decreased 25 cell numbers of WBC and lymphocytes, all of which are representative responses of individuals with depression,

thereby providing a normal immune system (*Ann N Y Acad Sci.* 917, 478-487 (2000)). These effects may be another therapeutic effect of antidepressants.

Recently, in the Western countries, medicinal herbal extracts have been recognized for their therapeutic effects and studied. With regard to depression, extracts of *Hypericum perforatum* (known also as St. John's wort) have been studied (*Neuropharmacology*, 1999, 21(2), 247-257; *Cochrane Database Syst Rev*, 2000, (2), CD000448; *Drugs Aging*, 2000, 16(3), 189-197).

According to a report that compared a *Hypericum perforatum* extract with imipramine for therapeutic efficacy on depression, the *Hypericum perforatum* extract has similar efficacy to imipramine in treating depression and has fewer side effects (*BMJ*, 2000, 321, 536-539). Also, the *Hypericum perforatum* extract has the potential to inhibit the activities of human cytochrome P450 enzymes (*J Pharmacol Exp Ther*, 2000, 294(1), 88-95).

The *Hypericum perforatum* extract contains a large number of structurally different compounds that directly or indirectly affect the central nervous system (CNS). That is, the *Hypericum perforatum* extract contains bioactive compounds, such as hypericin and hyperforin, and dimeric flavonoids, which are known to have antidepressive and apprehension-removing effects in animals and humans.

The action mechanisms of the constituents of *Hypericum perforatum* are as follows. Hypericin is proved to have the antidepressive effect in the presence of dimeric procyanidines contained in the *Hypericum perforatum* extract (Regensburg, Germany, V. Butterweck et.al., 45th Annual Congress of the Society for Medicinal Plant Research, 1997, Abstract No. 011). Hyperforin increases 5-HT (serotonin) levels in the hypothalamus and hippocampus, indicating that the antidepressive effect of hyperforin is associated with the serotonergic system (*J Pharm Pharmacol*, 2001, 53(5), 583-600; *Pharmacopsychiatry*, 2000, 33(2), 60-65). However, about 20% of depression patients are not treated with conventional antidepressants, and recently developed antidepressants such as SSRI have fewer side effects than other antidepressants, but they are still not negligible.

On the other hand, various depression animal models have been tried in the development process of antidepressants for treating depression. Strong stimuli such as intense foot-shock, cold water immersion and 48 h food/water deprivation were initially preferred, but, recently, preferred methods are to use weak repetitive stresses better capable of mimicking usual activities of modern people experiencing weak prolonged chronic stresses (*Psychopharmacology*, 1984, 83, 1-16). Among the recent methods, a chronic mild stress (hereinafter, referred to simply as "CMS") model, suggested

by Willner et al., has been approved as an excellent animal model of depression having reliability and validity (*Neuroscience and Biobehavioral Review*, 1981, 5, 231-246; *TIPS*, 1991, 12, 131-136).

5 "Mildly stressed rats" means that, when CMS-induced behavioral changes are observed for a prolonged administration period of weeks, the behavioral changes do not occur habitually, or habitual changes occur within a constant limitation (*Psychopharmacology*, 1997, 134, 319-320). In 10 general experiments, a variety of chronic weak stressors, such as overnight illumination, periods of food and/or water deprivation, cage tilt and change of cage mate, are used (*Psychopharmacology*, 1997, 134, 319-320). Repeated exposure of white rats to such stressors results in a significant 15 decrease in consumption of a sucrose solution, which is comparable to anhedonia, the representative symptom of depression of white rats. Upon no appropriate treatment, such decrease in consumption of a sucrose solution is known to last for several weeks after withdrawal of a CMS 20 procedure. Many antidepressants have been approved that they have effects of recovering the reduced sucrose intake induced by the CMS procedure to an original level (*Psychopharmacology*, 1992, 109, 433-438).

25 On the other hand, *Nelumbinis Semen* is the skinned ripe seed of lotus (*Nelumbo nucifera*), which has a green core. *Nelumbinis Semen* has no smell and a sweet, fresh and

slightly astringent taste.

Nelumbinis Semen contains a large quantity of starch and raffinose sugar, and is known to have the therapeutic effects of strengthening the spleen and stomach, alleviating 5 insomnia, whitening the skin, relieving inflammation and healing wounds in the skin. However, to date, there is no report of its ability to alleviate depression symptoms.

[Disclosure]

Therefore, the present invention aims to provide a 10 Nelumbinis Semen extract having antidepressive activity, a method of preparing the Nelumbinis Semen extract, and a pharmaceutical composition and a health food comprising the Nelumbinis Semen extract as an effective component.

Based on the fact that Nelumbinis Semen is used as a 15 Chinese traditional herbal medicine, the intensive and thorough animal behavioral research into the therapeutic effects of an extract of Nelumbinis Semen, conducted by the present inventors, resulted in the finding that the Nelumbinis Semen extract is superior in treating depression 20 to conventional antidepressants, *Hypericum perforatum* extract and fluoxetine (brand name: Prozac) one of SSRI, the recently most commonly used antidepressants, thereby providing a pharmaceutical composition for treating depression comprising the Nelumbinis Semen extract of the 25 present invention.

In addition, the molecular biological and biochemical research revealed the mechanism of the antidepressive action of the *Nelumbinis Semen* extract of the present invention, and resulted in the finding that the *Nelumbinis Semen* extract has another effect of normalizing immune functions, thereby providing a pharmaceutical composition for treating depression comprising the *Nelumbinis Semen* extract of the present invention.

Further, the animal behavioral research resulted in the finding that the *Nelumbinis Semen* extract of the present invention does not have the side effects that are observed upon application of conventional antidepressants.

Thus, in one aspect, the present invention provides a *Nelumbinis Semen* extract having antidepressive activity.

In another aspect, the present invention provides a method of preparing the *Nelumbinis Semen* extract.

In a further aspect, the present invention provides a pharmaceutical composition for treating depression, comprising the *Nelumbinis Semen* extract as an effective component.

In yet another aspect, the present invention provides a health food for treating depression, comprising the *Nelumbinis Semen* extract as an effective component.

Hereinafter, the present invention will be described in detail.

The present invention provides a *Nelumbinis Semen*

extract having antidepressive activity.

The *Nelumbinis Semen* extract of the present invention is prepared by a process including 1) extracting *Nelumbinis Semen* with an alcohol or an alcohol solution; 2) filtering 5 and concentrating the resulting extract; and 3) freeze-drying the resulting concentrate.

The alcohol or alcohol solution may be selected from the group consisting of 10-100% ethyl alcohol and 10-100% methyl alcohol. Preferred is 70-100% ethyl alcohol.

10 The extraction is carried out by cold extraction (maceration), under reflux conditions or by ultrasonic treatment. The ultrasonic extraction is preferred.

The present inventors investigated that the *Nelumbinis Semen* extract of the present invention has 15 antidepressive activity. After the *Nelumbinis Semen* extract of the present invention was administered, experimental animals were stressed by being exposed to bright light for a period of 48 hrs before a forced swim test. During forced swimming, the following behaviors were evaluated: struggling 20 time (time spent struggling, defined as strongly moving all four limbs), first latency (latency until first floating) and first rest duration (length of the first floating). As a result, the *Nelumbinis Semen* extract of the present invention displayed antidepressive activity and had a higher 25 antidepressive effect than a comparative group, *Hypericum perforatum* extract.

In addition, the present inventors compared the *Nelumbinis Semen* extract of the present invention with other Chinese medical herbs. The Chinese medical herbs as comparative subjects included *Rehmanniae Radix Preparat*, 5 *Lycii Fructus* and *Corni Fructus*, which strengthen body functions, and *Pinelliae Rhizoma* that has an expectorating effect (Table 1).

TABLE 1

Chinese medical herbs and their amount used

Chinese medical herb (Pharmaceutical name)	Amount
<i>Rehmanniae Radix Preparat</i>	500 g
<i>Corni Fructus</i>	500 g
<i>Lycii Fructus</i>	500 g
<i>Pinelliae Rhizoma</i>	500 g
<i>Nelumbinis Semen</i>	500 g
<i>Hypericum perforatum</i>	500 g

10 As a result, the *Nelumbinis Semen* extract of the present invention was found to have a higher antidepressive effect than extracts of *Rehmanniae Radix Preparat*, *Lycii Fructus*, *Corni Fructus* and *Pinelliae Rhizoma*.

15 In addition, the *Nelumbinis Semen* extract of the present invention was tested for its antidepressive activity and for overcoming sexual dysfunction, which is a representative side effect of conventional antidepressants. In this test using the aforementioned CMS model of depression in rats, being applicable to practical situations, where rats 20 were exposed to CMS to induce depression, the rats were

administered with conventional antidepressants, Prozac and a *Hypericum perforatum* extract, and the *Nelumbinis Semen* extract of the present invention, antidepressive effects and side effects of the administered drugs were evaluated by 5 objective comparison between test groups for behavioral changes including changes in weight, sucrose intake and physical activity in an open place. Also, a reduction in sexual behavior, which is a representative side effect of the SSRI depressants, was investigated by comparison between test 10 groups for mating behavior according to the above drugs. As a result, the *Nelumbinis Semen* extract of the present invention was found to have a higher antidepressive effect than the comparative drugs, *Hypericum perforatum* extract and Prozac, while not displaying reduced sexual behavior that is 15 a side effect of the above conventional antidepressive drugs, thereby indicating that the present *Nelumbinis Semen* extract does not have the side effects found upon the application of conventional antidepressive drugs.

Further, the mechanism of the antidepressive action 20 of the *Nelumbinis Semen* extract of the present invention was assessed according to the following molecular biological and biochemical methods.

First, according to a molecular biological method, after a depression-induced experimental animal was 25 administered with an effective amount of the *Nelumbinis Semen* extract of the present invention and the conventional

antidepressive drugs, the experimental animal with improved depression was incised at the cerebral frontal cortex. Total RNA was isolated from the cerebral frontal cortex, and double-stranded cDNA was synthesized using the isolated RNA and an oligo(dT)24-T7 primer. cRNA was synthesized by in vitro transcription using the synthesized cDNA, and was biotin-labelled and applied to an oligonucleotide microarray to determine gene expression patterns in each test group. As a result, the *Nelumbinis Semen* extract of the present invention was found, like the *Hypericum perforatum* extract and Prozac used as comparative drugs, to significantly increase expression levels of CREB, BDNF and trkB genes that are representative in vivo markers of antidepression.

Also, according to a biochemical method, the therapeutic efficacy of a candidate drug was primarily examined by measuring changes in 5-HT and norepinephrine (NE) levels in a chronic CMS model by microdialysis and HPLC-ECD. Catecholamine content was measured using an HPLC system equipped with an electrochemical detector. A mobile phase containing 0.05 M monobasic sodium phosphate, 0.1 N sodium acetic acetate and 1% methanol was adjusted to pH 4.4 with a phosphate buffer for HPLC. DA was composed of a Supelcosil LC-8-DB 3- μ m column (150 \times 4.6 mm, Supelco, Bellefonte, PA) protected by an LC-18 guard column. As a result, the *Nelumbinis Semen* extract of the present invention was found, like the *Hypericum perforatum* extract and Prozac

used as comparative drugs, to significantly increase levels of the 5-HT and NE neurotransmitters.

Secondarily, antidepressive effects were evaluated by an increase or decrease in receptor binding of a serotonin 5 2A receptor agonist, [³H]spiperone, in the rat brain frontal cortex, as follows. A behavioral test in rats was carried out for a period of three weeks, and the rats were administered with each drug. The brain frontal cortex was excised from the rats and frozen in liquid nitrogen. The 10 frontal cortex was cryo-sectioned into a horizontal form using a cryostat microtome, and each section was incubated in a [³H]spiperone-containing Tris-HCl buffer for two hours, dried, placed in an autoradiography cassette and exposed to an autoradiography film for four weeks in a refrigerator. 15 Then, the film was developed and scanned with a densitometer to measure light and dark intensity that corresponds to binding strength. After calibrated values were calculated using a standard scale bar, binding strength of each cryo-section was expressed as Ci/mg 20 tissue. When test groups were compared with a control group for an increase or decrease in receptor binding as an indication of antidepressive action, the *Nelumbinis Semen* extract of the present invention was found, like the *Hypericum perforatum* extract and Prozac used as comparative 25 drugs, to significantly increase the binding of the serotonin 2A receptor agonist, [³H]spiperone.

Thirdly, an increase in the antidepressive markers, CREB, BDNF and trkB proteins, was examined in the rat cerebral frontal cortex by Western blotting, as follows. A behavioral test in rats was carried out for a period of 5 three weeks, and the rats were administered with each drug. The brain frontal cortex was excised and centrifuged, and the supernatant was recovered. Each sample was mixed with a SDS-PAGE sample buffer to reduce proteins and boiled to denature proteins. Samples were loaded onto a SDS-PAGE 10 (sodium dodesyl sulfate poly acrylamide gel electrophoresis) gel and run at 100-200 V for about 1-2 hrs. The SDS-PAGE gel was transferred onto a PVDF or nitrocellulose membrane by electro-transferation. The membrane was incubated with a primary antibody for a target 15 protein, washed and incubated with a secondary antibody. Then, the target protein was detected by an enhanced chemiluminescent method. As a result, the *Nelumbinis Semen* extract of the present invention was found, like the *Hypericum perforatum* extract and Prozac used as comparative 20 drugs, to significantly increase protein levels of CREB, BDNF and trkB.

Fourthly, an increase in the antidepressive markers, CREB, BDNF and trkB proteins, was examined in the rat cerebral frontal cortex by 2-DE, as follows. A behavioral 25 test in rats was carried out for a period of three weeks, and the rats were administered with each drug. The brain

frontal cortex was excised and centrifuged, and the supernatant was recovered. Each sample was mixed with an IEF sample buffer and loaded onto an IEF gel. Proteins were separated according to isoelectric point by isoelectric focusing (IEF) in the first dimension and according to molecular weight by SDS-PAGE at 100-200 V for about 1-2 hrs in the second dimension. The gel was stained by a Gel-Code Blue staining method and evaluated for elevated or newly emerged proteins by the antidepressive substances. The elevated or newly emerged proteins by the antidepressive substances were subjected to mass spectrometry. As a result, the *Nelumbinis Semen* extract of the present invention was found, like the *Hypericum perforatum* extract and Prozac used as comparative drugs, to significantly increase protein levels of CREB, BDNF and trkB.

In addition, the *Nelumbinis Semen* extract of the present invention was evaluated to determine its ability to help overcome immune suppression caused by depression, by the following biochemical method.

First, changes in concentrations of cortisol, which is a representative marker to determine whether the immune suppression has been overcome, according to administration of the *Nelumbinis Semen* extract were examined, as follows. After urine samples were collected from rats,

(1) 1.0 ml of urine was placed into a tube with a cap, 2.0 ml dichloromethane was added to the tube, and the

tube was covered with the cap with caution;

(2) the mixture was vortexed for 5-10 min;

(3) the mixture was centrifuged at 1500×g (rpm), the upper layer was aspirated, and 50 µl of the lower layer 5 were aliquotted into coated tubes;

(4) samples were evaporated for dryness.

Urinary cortisol concentrations were measured as follows.

(1) The coated tubes were individually labeled with 10 NSB, Std (A-F), a control (CON6 No. 5) and sample numbers (for NSB, a green tube was used).

(2) 25 µl of each of NSB, Std (A-F), a control (CON6 No. 5) and samples was added to the corresponding tube (free cortisol was added to the completely dried coated 15 tube, and 25 µl of Std. A was added to the NSB tube).

(3) 1.0 ml of ¹²⁵I-cortisol was added to the tubes, followed by mixing.

(4) The tubes were incubated in a water bath at 37°C for 45 min and aspirated.

20 (5) After the content of the tubes was completely aspirated, radioactivity was measured for at least 1 min using a Y-counter.

As a result, the *Nelumbinis Semen* extract of the present invention was found, like the *Hypericum perforatum* 25 extract and Prozac used as comparative drugs, to restore cortisol concentrations to levels similar to a normal

group.

Secondarily, changes in concentrations of IL-2, which is a representative marker to determine whether the immune suppression has been overcome, according to administration 5 of the *Nelumbinis Semen* extract was examined, as follows.

After blood samples were collected from rats,

(1) 100 μ l of assay diluent QD6-23 were aliquotted onto a microplate strip;

10 (2) 50 μ l of each sample and a standard were aliquotted onto the strip;

(3) the strip was incubated with shaking at room temperature for 2 hrs;

(4) the strip was washed four times;

15 (5) 200 μ l of an IL-2 conjugate was aliquotted onto the strip, and the strip was incubated with shaking at room temperature for 3 hrs;

(6) 200 μ l of a substrate (luminol + hydrogen peroxide) was aliquotted onto the strip, and the strip was incubated at room temperature for 20-40 min;

20 (7) emitted light was measured using a luminometer; and

(8) IL-2 concentrations were determined using a standard quantitative curve.

As a result, the *Nelumbinis Semen* extract of the 25 present invention was found, like the *Hypericum perforatum* extract and Prozac used as comparative drugs, to restore

IL-2 concentrations to levels similar to a normal group.

Thirdly, changes in WBC cell number, which is a representative marker to determine whether the immune suppression has been overcome, according to administration 5 of the Nelumbinis Semen extract, was examined, as follows. After blood samples were collected from rats, cell volume, conductivity and light scattering were measured.

1) Cell volume

Cell volume was measured according to the Coulter 10 Principle, which is universally recognized as a reference method for sizing of volumes. An aperture between two electrodes is placed in a cell flow, and direct current flows between the electrodes. When WBC maintained at nearly natural states passes through the aperture, electrical 15 resistance to the direct current flow increases, thus generating a voltage pulse being in proportion to cell volumes. The size of the pulse is one of the distinct features allowing determination of the type of WBC. However, to distinguish between two types of cells with 20 similar size, a different phenotype allowing the distinction of two types of cells should be measured. For example, mature basophils and small lymphocytes are about 25 9-12 μm in diameter, and immature prolymphocytes and mature neutrophils are 12-14 μm in diameter. Different types of cells with similar size are difficult to determine based on only their size, and thus, in this case, conductivity and

light scattering are simultaneously measured.

2) Conductivity

Conductivity measurement is based on measuring cell contents using a high-frequency electromagnetic field. This 5 technology is a method of measuring cell contents, which started to be developed in the 1960s by Doctor Wallace Coulter, the founder of the Coulter Electronics Company, and was patented in USA in 1970. Doctor Coulter demonstrated that a high-frequency current is able to 10 penetrate the cell membrane. The current that has penetrated cells exists in specific forms according to the composition of the nucleus and granules and the internal chemical composition of cells. The application of this high-frequency electromagnetic field creates a novel 15 practical method capable of providing information about contents of cells. Doctor Coulter suggested a very important, new measurement item called "opacity", which has information about composition of the cytoplasm and the nucleus in an electromagnetic field where a high-frequency 20 current penetrates cells. "Opacity" is a conductivity signal reflecting the internal composition of cells, which is not influenced by cell size. "Opacity" measurement provided only by the Coulter Company is the most accurate and reliable technology for measuring cell contents. 25 Conductivity is useful for separating cells of similar size, but different internal composition. Only volume

measurement does not distinguish basophils from small lymphocytes. However, since conductivity measurement is carried out by measuring the difference between cell types in the ratio of the nucleus to the cytoplasm, granularity, etc., it is very useful for separating cell types.

5 3) Light scattering

In addition to volume and conductivity data, light scattering characteristics on the cell surface distinctly differ between cell types. Homogeneous light emitted from a 10 laser is collected using a lens and converted to a voltage pulse. Thus, light scattering is very useful for sorting cells according to the morphology and amount of granules.

WBC counting using the above methods resulted in the finding that the *Nelumbinis Semen* extract of the present 15 invention was found, like the *Hypericum perforatum* extract and Prozac used as comparative drugs, to restore the cell number of WBC to levels similar to a normal group.

Fourthly, changes in cell number of lymphocytes, which is a representative marker to determine whether the 20 immune suppression has been overcome, according to administration of the *Nelumbinis Semen* extract, was examined as follows. After blood samples were collected from rats, changes in lymphocyte cell number were examined by the following method.

25 1. Assay principle

When diluted blood corpuscles are suspended in an

electrolyte solution (Isoton sol.) that is a separate insulator, the electrolyte suspension of particles is suctioned for 4 sec through an aperture with a predetermined size. The aperture is placed between an 5 internal electrode and an external electrode, and a current flows between the electrodes. When particles pass through the aperture between the two electrodes, resistance increases while voltage decreases. The difference between decreased voltage and ground voltage is expressed as height 10 by a threshold circuit. The number of pulses indicates the number of particles, and the amplitude of pulses is in proportion to the volume of corpuscles.

2. Assay method: automatic analyzer
3. Machine used: ADVIA120, Bayer, USA
- 15 4. Reagents used:
 - 1) Isoton III, Beckman Coulter, USA
 - 2) Coulter clenz, Beckman Coulter, USA
 - 3) Lyse S III, Beckman Coulter, USA
 - 4) 4% sod. hypochloride-solution, Beckman Coulter,

20 USA

- 5) 4C-plus, Beckman Coulter, USA
- 6) Scatter Pak, Beckman Coulter, USA

As a result, the *Nelumbinis Semen* extract of the present invention was found, like the *Hypericum perforatum* extract and Prozac used as comparative drugs, to restore 25 the cell number of lymphocytes to levels similar to a

normal group.

In addition, the present invention provides a pharmaceutical composition for treating depression, comprising the Nelumbinis Semen extract as an effective component.

The present pharmaceutical composition for treating depression includes the Nelumbinis Semen extract as an effective component. The pharmaceutical composition may be administered orally or parenterally and may be formulated into typical pharmaceutical preparations.

That is, the Nelumbinis Semen extract of the present invention may be formulated into various formulations for oral and parenteral administration upon clinical application. In the formulation, diluents or excipients may be used, which are exemplified by fillers, thickeners, binders, humectants, disintegrators and surfactants.

Examples of solid formulations for oral administration include tablets, pills, powders, granules and capsules. The solid formulations may include, in addition to the Nelumbinis Semen extract, at least one excipient selected from among starch, calcium carbonate, sucrose, lactose, gelatin, etc. Also, the solid formulations may include, in addition to a simple excipient, a lubricant such as magnesium stearate or talc.

Examples of liquid formulations for oral administration include suspensions, internal solutions,

emulsions and syrups. The liquid formulations may include, in addition to commonly used simple diluents such as water and liquid paraffin, various excipients which are exemplified by humectants, sweeteners, aromatics and preservatives.

5 Examples of preparations for parenteral administration include sterile aqueous solutions, non-aqueous solutions, suspensions, emulsions, freeze-dried preparations and suppositories. In the formulation into 10 non-aqueous solutions and suspensions, propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable esters such as ethyl oleate may be used. As a base of suppositories, witepsol, macrogol, Tween 61, cacao fat, lanolin fat, glycerol and gelatin may be used.

15 The unit dose, may, for example, occurs one, two, three or four times, or a half, third or quarter of an individual dose. The individual dose preferably contains the amount of an effective drugs which is given in one administration and usually corresponds to a whole daily 20 dose or a half, third or quarter of the daily dose.

In the pharmaceutical composition for treating depression, an effective amount of the Nelumbinis Semen extract ranges from 30 to 700 mg/kg, and preferably 100 to 500 mg/kg, and may be administered once to six times daily. 25 The dosage for a specific patient may vary according to the patient's weight, age, sex, health state and diet,

administration duration, administration routes, excretion rates and severity of the illness.

When the *Nelumbinis Semen* extract of the present invention was orally, intraperitoneally and subcutaneously administered to white rats to evaluate its toxicity, 50% lethal dose (LD50) of the *Nelumbinis Semen* extract upon the intraperitoneal administration was higher than 20 g/kg. This result demonstrates that the *Nelumbinis Semen* extract is safe.

10 In addition, the present invention provides a health food for treating depression, comprising the *Nelumbinis Semen* extract as an effective component.

15 In the case of using the present extract as a food, the present extract may be added as it exists or in combination with other food or food ingredients, and may be used suitably according to general methods. Mixed amounts of effective components may be suitably determined according to the intended use (preventive, health or therapeutic purposes). Typically, the present extract may 20 be added in an amount of 0.01 to 1 wt%, and preferably 0.1 to 1 wt%, based on the total weight of raw materials used in preparing a food or drink. An effective amount of the present extract may be determined based on an effective amount of the pharmaceutical composition. When consumed for 25 a long period of time for health and sanitary purposes or health control, the present extract may be used in an

amount lower than the range. Also, it is apparent that the present extract can be used in an amount higher than the range because the effective component carries no safety risk.

5 The type of the food is not particularly limited. Examples of foods to which the present extract can be added include meats, sausages, breads, chocolates, candies, snacks, confectionary, pizza, instant noodles, other noodles, gums, dairy products including ice creams, various 10 soups, beverages, teas, drinks, alcoholic beverages and vitamin complexes, as well as traditional therapeutic preparations for use as an antianemic, a body function-strengthening agent, a skin whitening agent, and the like. In addition, the present invention may be used in various 15 prescriptions of Chinese medical decoctions, such as Reu Do Han Shao Tang, Quing Sin Shan Yao Tang and Tai Yin Tiao Wei Tang.

20 A better understanding of the present invention may be obtained, in conjunction with the accompanying drawings, through the following examples and experimental examples which are set forth to illustrate, but are not to be construed as the limit of the present invention.

[Description of Drawings]

25 Fig. 1 is a graph showing the struggling time measured in a forced swim test using white rats, in which,

for struggling time, a test group administered with a Nelumbinis Semen extract according to the present invention is compared to a control group and other test groups individually administered with a Rehmanniae Radix Preparat extract, a Corni Fructus extract, a Lycii Fructus extract, a Pinelliae Rhizoma extract and a Hypericum perforatum extract;

Fig. 2 is a graph showing the first latency time measured in a forced swim test using white rats, in which, for first latency time, a test group administered with a Nelumbinis Semen extract according to the present invention is compared to a control group and other test groups individually administered with a Rehmanniae Radix Preparat extract, a Corni Fructus extract, a Lycii Fructus extract, a Pinelliae Rhizoma extract and a Hypericum perforatum extract; and

Fig. 3 is a graph showing the first rest duration measured in a forced swim test using white rats, in which, for first rest duration, a test group administered with a Nelumbinis Semen extract according to the present invention is compared to a control group and other test groups individually administered with a Rehmanniae Radix Preparat extract, a Corni Fructus extract, a Lycii Fructus extract, a Pinelliae Rhizoma extract and a Hypericum perforatum extract.

[Mode for Invention]

EXAMPLE 1: Preparation of Nelumbinis Semen extract

500 g of Nelumbinis Semen dried powder was placed into a flask containing 1 L of 70% ethyl alcohol and subjected to ultrasonic extraction (Branson Co., USA) at room temperature for 10 min, and the supernatant was recovered. The pellet was further extracted with 85% and 100% ethylalcohol according to the same method as described above. The supernatants were pooled and filtered through a 10 gauze. The filtrate was concentrated using a vacuum filter (Eyela, Japan) and freeze-dried, thus yielding 95 g of a Nelumbinis Semen extract according to the present invention.

EXPERIMENTAL EXAMPLE 1: Evaluation of antidepressive 15 activity of the Nelumbinis Semen extract

The Nelumbinis Semen extract of the present invention was orally administered to postnatal 85-95-day Sprague-Dawley male rats. A comparative group was orally administered with a *Hypericum perforatum* extract. For 20 48 hrs before a forced swim test, the rats were stressed by being exposed to bright light (300 Lux).

A forced swim test was carried out as follows. On day 1, the white rats were placed into a cylindrical water bath (22 cm in diameter; 30 cm water depth) and forced to

swim for 10 min. On day 2, the rats were forced to swim for 5 min, and during this forced swimming, struggling time was measured.

As a result, during the forced swimming, the 5 comparative group administered with the *Hypericum perforatum* extract showed a non-significant increase in struggling time by 25.2% in comparison with a control group. In contrast, the *Nelumbinis Semen* extract significantly increased the struggling time by 43.9% (Fig. 10 1).

In addition, compared to the control group, administration of the *Hypericum perforatum* extract resulted in a non-significant increase in first latency time by 75.8%. In contrast, the *Nelumbinis Semen* extract 15 significantly increased the first latency time by 90.2% (Fig. 2).

Further, compared to the control group, in the comparative group administered with the *HYPERICUM perforatum* extract, no change was observed in first rest 20 duration. In contrast, in the group administered with the *Nelumbinis Semen* extract, the first rest duration decreased by 59.0% (Fig. 3).

These results indicate that the *Nelumbinis Semen* extract of the present invention has antidepressive 25 activity and is superior to the *Hypericum perforatum* extract used as a comparative group in counteracting

depression.

COMPARATIVE EXAMPLE: Comparison of the *Nelumbinis Semen* extract with other Chinese medicinal herbal extracts for antidepressive activity

5 The antidepressive effect of the *Nelumbinis Semen* extract was examined according to the same method as in Experimental Example 1 and compared with other Chinese medicinal herbal extracts of *Rehmanniae Radix Preparat*, *Corni Fructus*, *Lycii Fructus* and *Pinelliae Rhizoma*.

10 As a result, during the forced swimming, in comparison with a control group, administration of the *Rehmanniae Radix Preparat* and *Lycii Fructus* extracts resulted in a non-significant increase in struggling time by 15.2% and 4.9%, respectively, while administration of 15 the *Corni Fructus* and *Pinelliae Rhizoma* extracts resulted in a reduction of 3.9% and 1.1%, respectively. In contrast, the *Nelumbinis Semen* extract significantly increased the struggling time by 43.9% (Fig. 1).

20 In addition, compared to the control group, administration of the *Rehmanniae Radix Preparat*, *Corni Fructus* and *Pinelliae Rhizoma* extracts resulted in a non-significant increase in first latency time by 38.4%, 29.2% and 65.5%, respectively, while administration of the *Lycii Fructus* extract resulted in a reduction of 21.4%. In 25 contrast, the *Nelumbinis Semen* extract significantly

increased the first latency time by 90.2% (Fig. 2).

Further, compared to the control group, administration of the Rehmanniae Radix Preparat, Corni Fructus, Lycii Fructus and Pinelliae Rhizoma extracts 5 resulted in a reduction of 63.1%, 31.6%, 12.4% and 62.4%, respectively, in first rest duration. In contrast, administration of the Nelumbinis Semen extract resulted in a reduction of 59.0% in first rest duration (Fig. 3).

These results indicate that the Nelumbinis Semen 10 extract of the present invention has higher antidepressive activity than the Rehmanniae Radix Preparat extract.

EXPERIMENTAL EXAMPLE 2: Evaluation of acute toxicity of the Nelumbinis Semen extract upon oral administration to rats

An acute toxicity test was carried out using 6-week 15 specific pathogen-free (SPF) SD rats. The Nelumbinis Semen extract of the present invention was suspended in a 0.5% methylcellulose solution and orally administered to groups each consisting of five rats in a single dose of 5 g/kg, 10 g/kg and 20 g/kg. After administration of the extract, 20 death, clinical symptoms and weight change were observed, and a hematological test and hematobiochemical analysis were performed. Upon autopsy, abnormality in abdominal organs and chest organs was visually observed.

As a result, all rats administered with the extract 25 exhibited no particular clinical symptoms, no death, no

change in weight and no toxicity upon the hematological assay, hematobiochemical analysis and autopsy. As a result, the *Nelumbinis Semen* extract of the present invention exhibited no toxicity even at a dose of 10 g/kg in all 5 rats, and thus had a 50% lethal dose (LD50) higher than 20 g/kg upon oral administration. This result demonstrates that the *Nelumbinis Semen* extract is safe.

FORMULATION EXAMPLE 1: Preparation of soft capsules

Soft capsules were prepared according to a soft 10 capsule preparation method described in General Rules for Preparation in a guidebook, Korean Pharmacopoeia, using 100.0 mg per capsule of the *Nelumbinis Semen* extract prepared in Example 1, 175.0 mg of soybean oil, 45.0 mg of cera flava, 127.5 mg of hydrogenated palm oil, 21.0 mg of soybean phospholipids, 212.0 mg of gelatin, 50.0 mg of glycerin (gravity: 1.24), 76.0 mg of di-sorbitol, 0.54 mg of methyl-paraoxybenzoate, 0.90 mg of propyl-paraoxybenzoate, 0.56 mg of methylvanillin, and a proper 15 amount of yellow no. 203.

20 FORMULATION EXAMPLE 2: Preparation of tablets

100.0 mg of the *Nelumbinis Semen* extract prepared in Example 1, 90.0 mg of corn starch, 175.0 mg of lactose, 15.0 mg of L-hydroxypropylcellulose, 5.0 mg of polyvinylpyrrolidone 90 and a proper amount of ethanol were

homogeneously mixed, granulated by wet granulation, mixed with 1.8 mg of magnesium stearic acid, and forced into 400 mg tablets.

FORMULATION EXAMPLE 3: Preparation of capsules

5 100.0 mg of the *Nelumbinis Semen* extract prepared in Example 1, 83.2 mg of corn starch, 175.0 mg of lactose and 1.8 mg of magnesium stearic acid were homogeneously mixed, and filled into capsule shells at 360 mg per capsule.

FORMULATION EXAMPLE 4: Preparation of food and beverage

10 The present inventors prepared food and a beverage comprising the *Nelumbinis Semen* extract as an effective component, as follows.

<4-1> Preparation of chewing gum

15 Chewing gum was prepared according to a general method using 0.24-0.64% of the *Nelumbinis Semen* extract prepared in Example 1, 20% of gum base, 76.36-76.76% of sugar, 1% of a fruit aromatic and 2% of water.

<4-2> Preparation of ice cream

20 Ice cream was prepared according to a general method using 0.24-0.64% of the *Nelumbinis Semen* extract prepared in Example 1, 10.0% of milk fat, 10.8% of SNF (Solids Not Fat), 12.0% of sugar, 3.0% of starch syrup, 0.5% of an emulsion stabilizer (span), 0.15% of an aromatic (strawberry) and 63.31-62.91% of water.

<4-3> Preparation of beverage

A beverage was prepared according to a general method using 0.48-1.28 mg of the Nelumbinis Semen extract prepared in Example 1, 522 mg of honey, 5 mg of thioctic acid amide, 5 10 mg of nicotinic acid amide, 3 mg of riboflavin hydrochloride sodium, 2 mg of pyridoxine hydrochloride, 30 mg of inositol, 50 mg of orotic acid and 200 ml of water.

<4-4> Preparation of sausage

Sausage was prepared according to a general method 10 using 0.24-0.64% of the Nelumbinis Semen extract prepared in Example 1, 63.6% of pork, 27.5% of chicken, 3.5% starch, 1.7% of soybean proteins, 1.62% of edible salt, 0.5% of glucose and 0.94-1.34% of another additive (glycerin).

[Industrial Applicability]

15 As described hereinbefore, the Nelumbinis Semen extract of the present invention has very strong antidepressive activity, and Nelumbinis Semen, as the raw material of the Nelumbinis Semen extract, is a natural raw material used in Chinese medicine that is not harmful to 20 the body and is well absorbed by the body when used as a pharmaceutical composition for treating depression. Therefore, the Nelumbinis Semen extract is very useful for treating and preventing depression and various related diseases.

[CLAIMS]

~ [Claim 1]

A Nelumbinis Semen extract having antidepressive activity, which is prepared by extracting Nelumbinis Semen 5 with an alcohol or an alcohol solution.

~ [Claim 2]

The Nelumbinis Semen extract according to claim 1, wherein the alcohol or alcohol solution is selected from the group consisting of 10-100% ethyl alcohol and 10-100% 10 methyl alcohol.

~ [Claim 3]

The Nelumbinis Semen extract according to claim 2, wherein the alcohol or alcohol solution is 70-100% ethyl alcohol.

15 ~ [Claim 4]

A method of preparing the Nelumbinis Semen extract of claim 1, comprising:

- 1) extracting Nelumbinis Semen with an alcohol or an alcohol solution;
- 20 2) filtering and concentrating a resulting extract; and
- 3) freeze-drying a resulting concentrate.

[Claim 5]

The Nelumbinis Semen extract according to claim 4, wherein the extraction is selected from the group consisting of cold extraction (maceration), reflux extraction and ultrasonic extraction.

[Claim 6]

The Nelumbinis Semen extract according to claim 5, wherein the extraction is ultrasonic extraction.

[Claim 7]

10 A pharmaceutical composition for treating depression, comprising the Nelumbinis Semen extract of claim 1 as an effective component.

[Claim 8]

15 A health food for treating depression, comprising the Nelumbinis Semen extract of claim 1 as an effective component.

AMENDED CLAIMS

[received by the International Bureau 20 December 2004 (20.12.2004);
original claims 5 and 6 amended; 1 to 4 and 7-8 claims unchanged (1 page)]

+ STATEMENT**【Claim 5】**

The method according to claim 4, wherein the extraction is selected from the group consisting of cold extraction (maceration), reflux extraction and ultrasonic extraction.

5

【Claim 6】

The method according to claim 5, wherein the extraction is ultrasonic extraction.

【Claim 7】

10 A pharmaceutical composition for treating depression, comprising the *Nelumbinis Semen* extract of claim 1 as an effective component.

10

【Claim 8】

15 A health food for treating depression, comprising the *Nelumbinis Semen* extract of claim 1 as an effective component.

15

Statement Under Article 19

This Statement refers to the amendment under Article 19. The amendment should be filed on the basis of the below reason.

Claim 5 is dependent on claim 4, but claim 5 do not refer to claim 4 correctly. As claim 4 is related to a method of preparing the Nelumbinis Semen extract, the claim 5 should be amended as “The method according to claim 4,”, instead of “The Nelumbinis Semen extract according to claim 4.”

Further, claim 6 is dependent on claim 5, but claim 6 do not refer to claim 5 correctly. As claim 5 is related to a method of preparing the Nelumbinis Semen extract, the claim 5 should be amended as “The method according to claim 5,”, instead of “The Nelumbinis Semen extract according to claim 5.”

Please be assured that the amendments will not have any impact on the description and the drawings.

1/2

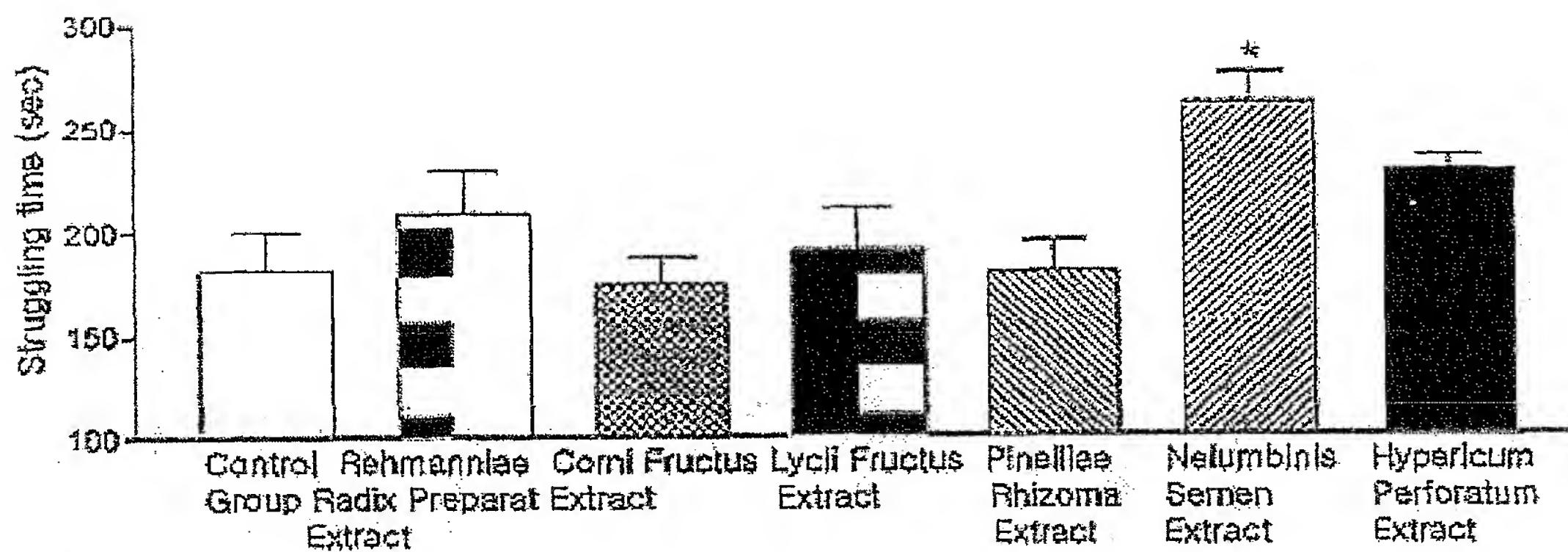


FIG. 1

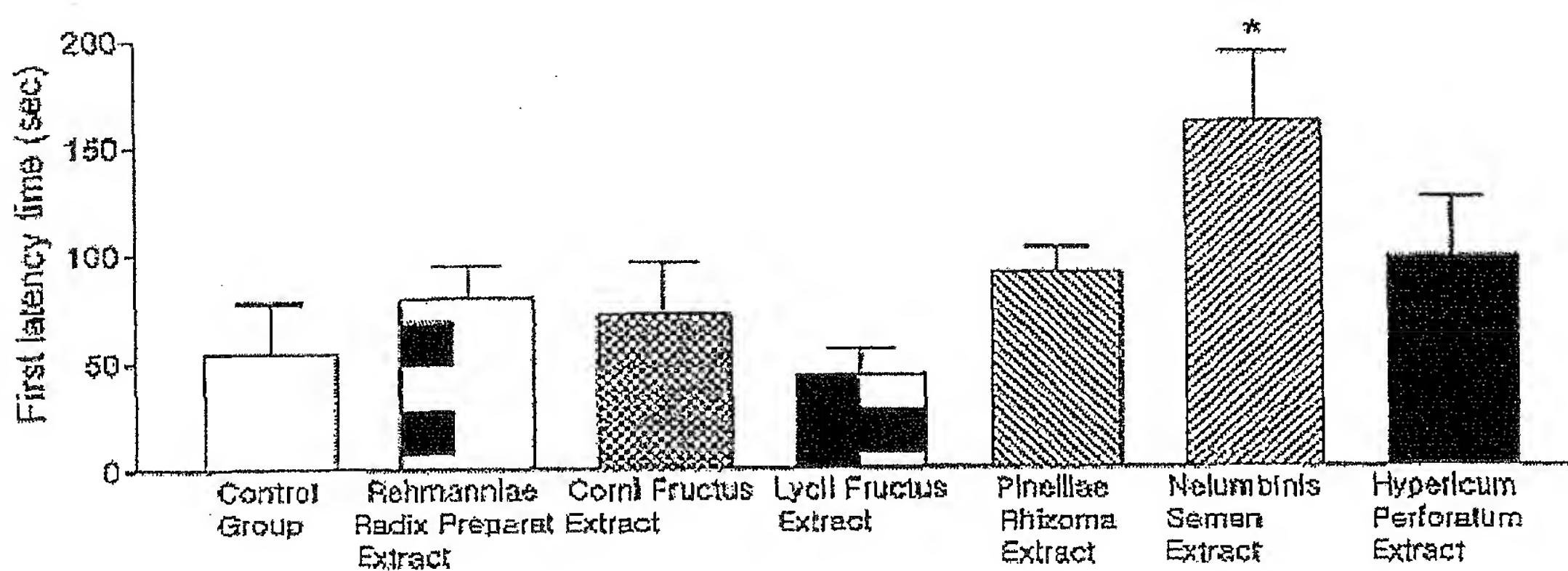


FIG. 2

2/2

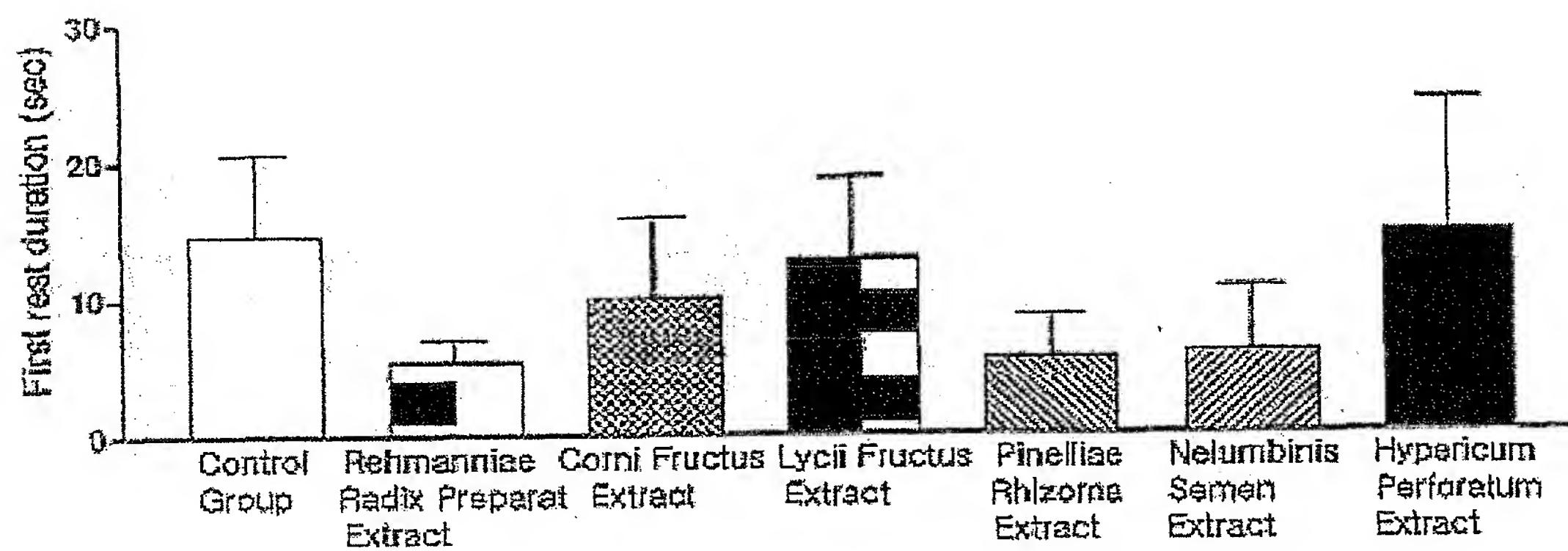


FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2003/001743

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 A61K 35/78

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K 35/78, A23L 1/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
KOREAN PATENTS AND APPLICATIONS FOR INVENTIONS SINCE 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubMed on-line

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E, X	KR 2003-0079104 A (PURIMED INC.), 10 October 2003 See entire document	1-8
E, A	KR 2004-0026175 A (KYUNGHEE UNIVERSITY), 30 March 2004 See entire document	1-8
A	KR 2002-0015540 A (KIM, SK), 28 February 2002 See abstract	1-8
A	MUKHERJEE, PK et al. 'Studies on psychopharmacological effects of <i>Nelumbo nucifera</i> Gaertn. rhizome extract' In; <i>J. Ethnopharmacol.</i> 1996; 54: 63-7	1-8
A	SHOJI, N et al. 'Asimilobine and lirinidine, serotonergic receptor antagonists, from <i>Nelumbo nucifera</i> ' In; <i>J. Nat. Prod.</i> 1987; 50(4): 773-4	1-8

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier application or patent but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "F" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

11 MAY 2004 (11.05.2004)

Date of mailing of the international search report

11 MAY 2004 (11.05.2004)

Name and mailing address of the ISA/KR



Korean Intellectual Property Office
920 Dunsan-dong, Seo-gu, Daejeon 302-701,
Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

YEO, Ho Sup

Telephone No. 82-42-481-5627



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2003/001743

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- 1) Claims 1-7 are directed to a pharmaceutical composition.
- 2) Claims 1-6 and 8 are directed to a health food.

Since the abovementioned groups of claims do not share any of the technical features identified, a technical relationship between the inventions does not exist.

Accordingly the claims do not relate to one invention or to a single inventive concept, a priori.

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any addition fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2003/001743

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
KR 2003-0079104 A	10/10/2003	NONE	
KR 2004-0026175 A	30/03/2004	NONE	
KR 2002-0015540 A	28/02/2002	NONE	

